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(54) **Stabilization of highly purified proteins.**

(57) A carbohydrate, such as mannitol or Dextran-70, is added during the purification of a protein, such as Factor VIII:C. The carbohydrate stabilizes the protein, even when the protein is 60-99% pure. The carbohydrate is added across various purification steps and is present in the final container so as to stabilize the protein during freeze-drying and reconstitution.

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STABILIZATION OF HIGHLY PURIFIED PROTEINS

BACKGROUND OF THE INVENTION

1. Field of the Invention:

The present invention relates to protein products which are highly purified and biologically active, such as therapeutic proteins intended for human use. In particular, the present invention relates to the use of carbohydrates to stabilize zymogens, such as Factor VIII:C, and monoclonal antibodies.

2. Description of the Related Art:

The prior art may be thought of as falling into three categories: (1) the stabilization of unpurified proteins, such as Factor VIII concentrates; (2) the stabilization of highly purified proteins; and (3) other uses of carbohydrates, such as dextran or sorbitol.

(1) Rock 4,359,463 discloses stabilization of Factor VIII activity in whole blood or blood plasma by addition of a calcium chelating anticoagulant such as acid citrate dextrose (ACD) or citrate phosphate dextrose (CPD). Heparin is also added.

Mathews et al. 4,743,680 disclose a method of purifying Factor VIII from plasma using a chromatography column exemplified by QAE-Sephadex, polyelectrolyte and aminohexyl-Sepharose resins. Sugars, polyhydric alcohols, and amino acids enhance the selective binding of proteins to these ion exchange chromatography media. In one example, 1M sorbitol is contained in a buffer which is added to the sample at the $Al(OH)_3$ absorption stage (Col. 5, 1. 63). Sugars, including sucrose, mannose and dextran can be used instead of a polyhydric alcohol (Col. 15, 1. 7).

Rasmussan et al. 4,650,858 disclose preparation of a high purity (50 u/mg) Factor VIII preparation using a PEG precipitation step which includes the use of "salting-in agent" such as an amino acid or a carbohydrate. Suitable carbohydrates may include sugar alcohols and oligosaccharides.

Thomas 4,089,944 discloses a method of improving the solubility of a Factor VIII composition by the addition of 0-5 grams of dextrose per 100 ml of reconstituted solution. Disaccharides and short chain oligosaccharides (dextrins) can also be used.

Winkelman 4,789,733 discloses the recovery of Factor VIII from plasma through precipitation with a sulphated polysaccharide, i.e. heparin. Dextran sulphate reportedly can also be used.

Schwinn et al. 4,297,344 disclose a heat treatment for plasma proteins including Factor VIII. An amino acid and a monosaccharide, oligosaccharide or sugar alcohol are added to an aqueous solution in order to stabilize the protein(s) against the heat. 20 to 60 w/w % sucrose is preferred.

Fernandes et al. 4,440,679 disclose heat treatment of plasma proteins (including Factor VIII) in the presence of a polyol as the sole stabilizing agent. The preferred "polyol" is sucrose; higher molecular weight polyols such as dextran, starch or glycogen are not preferred for use (Col. 6, 1. 44) with the disclosed process.

EP 077,870 discloses the heat treatment of Factor VIII in the presence of 10% (w/v) or more of at least one stabilizer selected from the group consisting of neutral amino acids, monosaccharides, oligosaccharides and sugar alcohols and 10% or more of an auxiliary stabilizer of a carboxylic acid 3-10 carbon atom.

Naito et al., 4,446,134 disclose heat treatment of Factor VIII concentrates with 10-60% (w/v) of a stabilizer selected from the group consisting of neutral amino acids, monosaccharides, oligosaccharides or sugar alcohols. A carboxylic acid is used as an auxiliary stabilizer.

Ng et al., Thromb. Res. 39:439-447 (1985) disclose a process for heat treating Factor VIII in solution which uses sucrose as a stabilizer.

Foster et al., Vox Sang. 55:81-89 (1988) discuss the stability of Factor VIII during the processing of Factor VIII:C from human plasma. Losses over the processing steps were minimized by the addition of calcium. The calcium prevented inactivation of Factor VIII:C caused by sodium citrate, which is added during the fractionation process. Various stabilizing agents (e.g. heparin, glycine and sugars) are referenced.

(2) with regard to highly purified, therapeutically active proteins, it is generally accepted that recombinant DNA derived proteins, regardless of whether from a bacterial, yeast or mammalian cell source, as well as monoclonal antibodies, must be purified to at least 99% purity if the product is intended for human use. Generally, human serum albumin is added to stabilize such purified proteins against denaturation or

aggregation which may occur in a highly pure solution. The use of human serum albumin as a stabilizer has one drawback with regard to cost. In addition, albumin is not wholly satisfactory in conjunction with the use of therapeutic proteins, where the perceived benefit of using recombinant DNA derived proteins instead of plasma-derived proteins is greatly diminished when plasma-derived albumin is included in the final container formulation.

Plan et al. 3,992,367 disclose a purified albumin heated in the presence of caprylic acid, which is added for heat stabilization.

Vemura et al. 4,361,652 disclose the heat stabilization of plasma-derived plasminogen with inorganic salts such as NaCl.

Kwan 4,496,537 discloses the stabilization of alpha interferon preparations over the lyophilization step through the addition of glycine or alanine. 1-10 mg/ml albumin is also added as a stabilizer.

Mori et al. 4,505,893 disclose a purified plasminogen activator stabilized with 1-10 mg/ml of albumin.

Murakimi et al. 4,552,760 disclose a method for stabilizing tissue plasminogen activator through the addition of gelatin.

It is also known that albumin stabilizes drugs and organic compounds such as thromboxane. See Folco, et al. FEBS Letters 83(2):321-324 (1977). In addition, highly purified plasma-derived Factor VIII:C products are known to be stabilized with albumin.

(3) With regard to the use of carbohydrates, Battle et al., Thromb. Haemot. 54(3):697-699 (1985) disclose that infusion of 500 ml of 6% Dextran 70 (70,000 M.W.) into normal adults leads to an apparent decrease in vWF multimers. The maximum effect was at about 6 hours. Factor VIII:C activity also decreased at about 6 hours.

Artusson et al., J. Pharm. Sci. 73(11):1507-1513 (1984), report that biodegradable microparticles of cross-linked starch may be used as carriers of proteins and low molecular weight drugs. The starch used was acrylolated maltodextrin or acrylolated hydroxyethyl starch. The particles were used to immobilize carbonic anhydrase, and it is concluded that the particles may be useful as a drug carrier system.

Aberg et al., Ann. Surg. 189(2):243-247 (1979), describe a study on the effect of an infusion of 500 ml of 6% Dextran 70 on Factor VIII activity. The study is an attempt to clarify the mechanism of the effect of dextran on platelet function. Factor VIII antigen levels significantly decreased after dextran infusion.

Raasch, Am. J. Hosp. Pharm. 36:89-91 (1979), reports that infusion of Dextran 70 may, in some cases, result in reduced Factor VIII activity.

In summary, the above-described prior art discloses the use of various carbohydrates which may be added to final container protein solutions of intermediate purity for stabilization of the protein during heat treatment. Highly purified cell culture derived proteins are stabilized across the lyophilization step with albumin or compounds such as gelatin, glycine, alanine, NaCl, etc. Carbohydrates of high molecular weight may be used as drug carriers.

Accordingly, it is an object of the present invention to provide a compound which may be added as a stabilizer during the normal purification steps of cell culture derived protein and which will maintain the stability, i.e., molecular integrity and biological activity, of the purified protein.

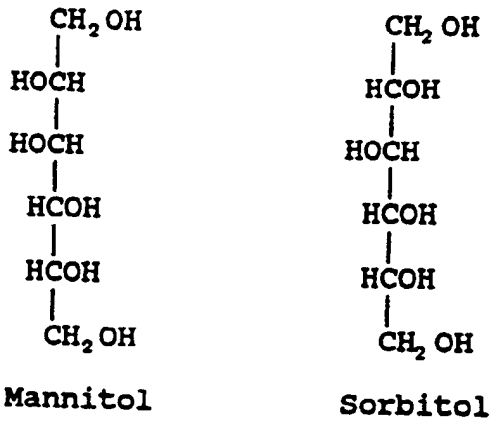
It is also an object of the invention to provide a stabilizer which is known to be acceptable for intravenous use, but is not derived from a biological (i.e. plasma) source.

It is also an object of the present invention to provide a stabilizer which can be retained across the purification process as part of a final product formulation.

SUMMARY OF THE INVENTION

The present invention comprises the use of selected carbohydrates for the stabilization of proteins in highly purified form (i.e. at least 90% total protein). High molecular weight carbohydrates suitable for use in the present invention are glucose polymers and include, dextrans, starches, cellulose derivatives, and most preferably, dextran. An important aspect of the use of dextrans in the molecular weight range of 25,000 to 100,000 daltons (25-100 kd) is that such compounds can be selected to approximate th molecular weight of the protein to be purified. Sugar alcohols, i.e. alcohols derived from 5- or 6- carbon sugars, such as sorbitol or mannitol, may also be used in the present invention. An important advantage in the use of mannitol is that mannitol is expected to be highly suitable for injection into human beings.

Sorbitol or mannitol, M.W. 182, may be represented as follows:



These sugar alcohols are re-added at various process steps but are relatively inexpensive and have high physiological safety and toxicity profiles.

An important aspect of the present invention is that the protein to be stabilized has molecular integrity which is required to produce a measurable biological activity. A preferred protein for use with the present invention is Factor VIII:C, a coagulation factor which is extremely large in its native state (approximately 300 kD) and is extremely labile, being detected in highly purified preparations as a mixture of peptides of 40-210 kD. The integrity of the Factor VIII:C may be assessed by examination of this peptide mixture on gels. With regard to monoclonal antibodies, biological activity may be measured by binding activity and integrity may be measured by aggregation. Aggregation of IgG monomers makes such proteins unsuitable for intravenous administration. Similarly, such monomers may separate into their respective heavy and light chain or otherwise break down into partially active or inactive fragments.

The present invention contemplates the use of the following stabilizers: dextran, 1-50 mg/ml; and mannitol or sorbitol, 1-10 mg/ml preferably 5-10 mg/ml.

The present stabilizers have been shown to be at least equivalent to albumin with regard to stabilizing effect. The present stabilizers have been shown to be superior to other compounds, such as glycine, which have previously been used for stabilization of highly purified proteins.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Materials and Methods

Recombinant Factor VIII:C (rFVIII) was produced from BHK-21 cells transfected with the cDNA coding for human Factor VIII (Vehar et al., Nature 312:337-342 (1984), Wood et al., Nature 312:330-337 (1984)).

Clarified tissue culture fluid was applied to a DEAE-Sepharose column that had been equilibrated, washed and eluted as follows: Equilibration and wash were carried out with 0.02M imidazole, 0.01M $CaCl_2$, 0.075M NaCl, pH 6.9. Elution was carried out with 0.02M imidazole, 0.01 $CaCl_2$, 0.25M NaCl, pH 6.9.

Immunoabsorption on a monoclonal antibody (MAb) binding to rFVIII (C7F7) was carried out with immobilized C7F7 antibody. (See Eaton et al., Biochemistry 25(2): 505-512 (1986), EPO 160 457).

The column was equilibrated, washed and eluted as follows: Equilibration and wash were carried out with 0.02M imidazole, 0.01M $CaCl_2$, 0.5M NaCl, pH 6.9. Elution was carried out with 0.02M imidazole, 1M $CaCl_2$, 0.7M NaCl, pH 6.9.

C7F7 eluate was applied to a gel filtration column, Pharmacia. Equilibration and wash were carried out with 0.02M imidazole, 0.01M $CaCl_2$, 0.05M NaCl, .01% Tween 80, pH 6.9.

Diafiltration and ultrafiltration were carried out using an Amicon stirred cell with a YM-30 membrane.

Glycine USP was obtained from Sigma Chemical Corp.; $CaCl_2$ was obtained from Fisher Scientific; NaCl was obtained from J. T. Baker, USP; albumin was pharmaceutical grade albumin 25%, Plasbumin, Cutter Biological; and sorbitol and mannitol were reagent grade obtained from Baker Chemical Co.

Dextran 70 is defined as that fraction of dextran, a branched polysaccharide composed of glucose units, having an average molecular weight of 70,000. (Physician's Desk Reference, 1989, 43rd ed., p. 1630). Dextran 70 was obtained from Pharmacia.

Factor VIII:C activity was measured by the one stage clotting assay, using the WHO Mega standard. One unit of activity is defined as the coagulation activity of Factor VIII (F.VIII) in one ml of normal plasma. Specific activity is defined as units of activity per total protein mass. Factor VIII may be designated as essentially pure at 5,000 IU/mg specific activity. Purity may also be defined antigenically. F.VIII may be stabilized at greater than about 60% purity by addition of the present carbohydrates.

Example 1

This Example illustrates a study of various compounds used in stabilizing F.VIII across the freeze-drying step.

F.VIII from clarified tissue culture fluid (tcf) was eluted from a DEAE ion exchange column. A DEAE pool was obtained and the protein was purified through an anti-F.VIII monoclonal antibody designated C7F7 (40 ml column volume). To this eluate was added glycerol to 15%. Then, after G-25 gel filtration desalting (on a 600 ml column), the pool was adjusted to contain 0.275 M glycine. Then, three 45 ml aliquots were taken out. To aliquot number one, albumin was added to 1 mg/ml. This was a control or standard in each set of experiments. The recovery of the F.VIII solution across each step was set at 1.0 based on the albumin control. To the second aliquot, and/or to a third aliquot, another excipient and/or another concentration to be tested was added. These included dextran (M.W. 10,300) sorbitol, mannitol, dextrose, and methyl cellulose. No addition was also a control.

Then each 45 ml aliquot was concentrated 3-fold by ultrafiltration (uf) with a stirred cell and an YM-30 membrane. The 15 ml was then freeze-dried in 5 ml aliquots. Assays taken after concentration and after freeze drying are compared in the table to the albumin control (which is set at 1.0).

The data in Table 1 below indicate that no addition of stabilizer gives almost no recovery, and that mannitol, sorbitol, and dextran all help preserve the Factor VIII activity across the freeze dry step. The stabilizers illustrated compare favorably to albumin. It should also be noted that these results were obtained with the use of 15% glycerol, which is also considered a stabilizer. Results superior to the use of glycerol above were obtained.

Table 1

Dextran	After uf	After freeze-dry
50 mg/ml	1.22	1.64
10	2.94	1.2
5	1.45	1.59
3	1.14	
2	1.5	0.81
1	0.91	0.61
1	0.64	0.33
Sorbitol	0.45	0.96
5 (0 mg/ml)	0.72	0.72
1	0.99	0.54
Mannitol		
10 mg/ml	1.14	1.28
5	1.19	1.46
Methyl Cellulose		
10 mg/ml	1.31	N.D.
5	0.79	N.D.
Control no addition	---	0.05; 0.3; 0.1
Dextran 70		
5 mg/ml	1.31	2.31
4	1.64	2.01
3	1.32	2.13
2	1.34	1.61
1	0.85	1.30

Example 2

This Example utilizes Dextran 70, identified as a freeze-dry stabilizer in the preceding example.

60 ml of C7F7 eluate were obtained after DEAE chromatography of clarified tissue culture fluid, as in the previous example. Glycerol was added to C7F7 eluate to a final concentration of 15%. This was divided into 2 aliquots. To one aliquot albumin was added to a final concentration of 1 mg/ml, to the other Dextran 70 was added to a final concentration of 1 mg/ml. Each aliquot was applied to a 150 ml G-25 gel filtration column as described above to change the buffer and salt concentration to a value which allow F.VIII binding to the next C7F7 column. Each pool from this column was then applied to another 8 ml C7F7 column. The first aliquot produced 13 ml of eluate. 52 µl of 25% albumin was added to yield 1 mg/ml of albumin. The second eluate produced 23 ml of eluate; 442 µl of 6% Dextran 70 was again added for a final dextran concentration of 1 mg/ml. Glycerol was added to each to a final concentration of 15%. Then, a second G-25 column was run to change buffer so that it would be compatible with and bind to the DEAE Sepharose. In the albumin control (first aliquot), 19 ml of eluate resulted. 0.3 ml of 25% albumin was added to achieve a final concentration of 4 mg/ml. Likewise, the pool from the second aliquot had 1.5 ml of 6% Dextran added to a 21.5 ml pool to achieve a final concentration of 4 mg/ml dextran. Each sample was then diafiltered with a YM 30 membrane against 5 volume changes of buffer and adjusted by concentration or dilution to 20 ml. This material was then freeze dried in a standard laboratory freeze-drier (Virtis Unitrap II at -70° C, 0.1 mm Hg).

The results are shown below in Table 2.

Table 2

	Albumin			Dextran		
	u/ml	ml	Yield	u/ml	ml	Yield
1st C7F7	216.4	30.0	100.0%/100.0%	216.4	30.0	100.0%/100.0%
1st G-25	76.6	70.0	82.6%/ 82.6%	88.6	64.0	87.3%/ 87.3%
2nd C7F7	335.3	15.0	93.8%/ 77.5%	213.1	26.5	99.6%/ 87.0%
2nd G-25	57.7	70.0	80.3%/ 62.2%	62.6	59.0	65.4%/ 56.9%
Overnight	69.1	70.0	119.8%/ 74.5%	76.1	59.0	121.6%/ 69.2%
3rd DEAE	178.7	19.5	72.0%/ 53.7%	142.4	23.0	73.0%/ 50.5%
Diafilt.	68.2	20.0	39.1%/ 21.0%	71.3	20.0	43.5%/ 22.0%
Freeze-dry	56.8		83.3%/ 17.5%	68.7		96.4%/ 21.2%

Table 2 yields are expressed as step yield/cumulative yield. In each experiment, the stabilizer was at 1 mg/ml during processing, which was increased to 4 mg/ml for freeze drying. Dextran compares favorably with albumin during both processing and freeze-drying.

Example 3

This example reproduces the experiment of Example 2, but with different stabilizer concentrations.

In this experiment, two 31.5 ml aliquots of 1st C7F7 eluate were obtained, and processed to the 3rd DEAE eluate stage with either dextran or albumin. Then from Aliquot 1, 22 ml of eluate were mixed with .44 ml of 25% albumin to give 5 mg/ml. The Aliquot 2 eluate was mixed with 1.73 ml of 6% dextran (26 ml of eluate) to give 4 mg/ml. Each was then diafiltered vs. 5 volumes of buffer, concentrated to 15 ml each and freeze-dried. The data are shown in Table 3:

Table 3

	Albumin			Dextran		
	u/ml	ml	Yield	u/ml	ml	Yield
1st C7F7	114.1	31.5	100.0%/100.0%	125.5	31.5	100.0%/100.0%
1st G-25	51.1	84.0	119.4%/119.4%	54.9	70.0	97.2%/ 97.2%
2nd C7F7	232.8	17.5	94.9%/113.4%	128.9	32.0	107.3%/104.3%
2nd G-25	54.0	75.0	99.4%/112.7%	44.1	79.0	84.5%/ 88.1%
Overnight	51.8	75.0	95.9%/108.1%	51.3	75.0	110.4%/ 97.3%
3rd DEAE	121.0	22.5	70.1%/ 75.8%	120.3	27.7	86.6%/ 84.3%
Diafilt.	184.6	15.0	101.7%/ 77.0%	134.4	15.5	62.5%/ 52.7%
Freeze-dry	130.0		70.4%/ 54.3%	99.0		73.7%/ 38.8%

These data show the comparability of dextran and albumin at 4 mg/ml and 5 mg/ml, respectively.

Example 4

This Example is directed to final container formulations.

The following Table 4 shows a representative final container formulation wherein albumin has been replaced by dextran. Other excipients in other concentrations can be used. Excipients and stabilizers are adjusted in the final bulk stage, just prior to container filling. The containers are then freeze dried.

Table 4

Ingredient	With albumin	Without albumin
Factor VIII	100 u/ml	100 u/ml
Glycine	0.275M	0.275M
$CaCl_2$	0.0025M	0.0025M
NaCl	0.1M	0.1M
Albumin	5-8 mg/ml	0
Dextran-70	0	1-5 mg/ml

Another final container formulation includes, in place of albumin or dextran, mannitol or sorbitol at 5-8 mg/ml. The final container formulation may contain as much as 50 mg/ml of stabilizer.

Mannitol is considered superior to sorbitol for this application because sorbitol is metabolized to produce fructose and may cause fructose intolerance in some patients.

Example 5

In this series of experiments mannitol was employed in connection with the processing of a second DEAE pool, which was placed on a C7F7 column. To the eluate (10-15 ml), 5 mg/ml mannitol was added. This eluate was applied to a CL6B gel filtration column, pooled, ultrafiltered and diafiltered (UF/DF) and freeze-dried. In some experiments, mannitol was also added to 5 mg/ml to the diafiltration buffer and/or CL6B wash buffer. The results are shown in Table 5 below.

Table 5

	Exp. A	Exp. B	Exp. C
Starting material	100/100	100/100	100/100
C7F7	81/81*	124/124*	NA/NA*
CL6B	62/50*	40/50	NA/65*
UF/DF	79/40*	6/3	25/16
Freeze-dry	67/27	NA	NA

Results are expressed as percentage step yield/cumulative yield. An asterisk (*) indicates addition of mannitol at that step. Yields in excess of 100% result from assay variability.

The results show that addition of mannitol to the CL6B wash buffer and the uf/df buffer is essential to process yield. Addition of mannitol to additional buffers improves yields, and addition of mannitol to each sequential step dramatically improves yields over the process. The mannitol yield in this series of experiments was comparable to the yield observed in comparable experiments wherein albumin was added at the C7F7 step. Experiments with sorbitol produced results comparable to those with mannitol in Exp. A. Representative yields with sorbitol were: starting material, 100/100; C7F7, 91/91*; CL6B, 86/77*; UF/DF, 88/68*.

Example 6

A purified murine IgG monoclonal antibody against tumor necrosis factor was studied by HPLC for molecular integrity. All samples were prepared with glycine .27M at pH 4.0.

The following Table shows the molecular size distribution after freezing and thawing. Numbers are % protein in various HPLC peaks, which are denominated high M.W. (aggregates), IgG, and low M.W.

(fragments).

Table 6

Sample	High M.W.	IgG	Fragments
Liquid Control	3	97	-
.27 M glycine	8	90	2
dextran 5 mg/ml	3	97	-
sorbitol 10 mg/ml	3	97	-
Liquid Control	2	96	2
.27 M glycine	11	87	2
sorbitol 1 mg/ml	7	91	2
sorbitol 5 mg/ml	4	96	2
sorbitol 10 mg/ml	2	96	2
dextran 1 mg/ml	9	88	3
dextran 5 mg/ml	5	92	3
mannitol 5 mg/ml	7	90	3

Liquid controls were not frozen. Highest monomer levels (IgG) were found at dextran 5 mg/ml and sorbitol at 5 or 10 mg/ml. Sorbitol or dextran at 1 mg/ml had little or no protective effect.

It is important to note that all samples contained 0.27M glycine, which has also previously been described as a stabilizer. Significantly superior results were achieved when the compounds of the present invention, were added to samples already containing glycine.

Discussion

It is well known that highly purified proteins are highly unstable. In these Examples, the rF.VIII used has generally a specific activity of 2,000-5,000 at the immunoabsorption step, corresponding to a purity of roughly 60-99% pure. The stabilizers of the present invention may be used with other highly purified proteins having biological activity, such as monoclonal antibodies. Other purified, biologically active proteins to which the present invention may be applied may include, for example, the following rDNA derived proteins: alpha, beta and gamma interferon, tissue-type plasminogen activator, tumor necrosis factor, human growth hormone, erythropoietin, colony stimulating factors, Factor IX, Protein C, superoxide dismutase, IL-2, epidermal growth factor, etc.

The process steps disclosed herein are merely representative of typical protein purification steps, i.e. anion exchange chromatography, affinity chromatography, gel filtration, ultrafiltration, etc. Other steps such as Protein A chromatography (for purifying IgG's), HPLC, etc. could also be used. No particular significance is attached to the use of the anti-F.VIII:C C7F7 immuno-purification monoclonal antibody used; other MAb's could be used as well.

Among the particular stabilizers used, mannitol is most preferred because it is expected to be well tolerated in human subjects. Dextran or the like may be preferred because its high molecular weight allows it to be carried through the purification process with a high M.W. protein of interest, e.g., F.VIII:C. F.VIII:C generally has a range of molecular weights in its purified form, with 80-90 kD chains being predominant. Thus, the dextran used, with 70 kD M.W. approximately matches the M.W. of the protein chains to be stabilized.

Claims

1. A method of purifying a protein to at least 60 % purity, comprising the steps:
(a) adding to an impure solution of said protein a carbohydrate selected from the group consisting of sugar alcohols and glucose ploymers having an M.W. of 25-100 kd;
(b) passing the solution from step (a) through at least one chromatography step, selected from the group consisting of ion exchange chromatography, affinity chromatography, and size exclusion chromatog-

raphy, whereby said protein achieves at least 60% purity; and
(c) adding said carbohydrate to a final container solution for freeze drying.

2. The method of claim 1 wherein said carbohydrate is dextran or mannitol.

3. The method of any of claims 1 and 2 wherein said protein is Factor VIII :C.

4. A composition, comprising:
(a) a therapeutically active protein having a purity of at least 60%;
(b) 1-5% excipients selected from the group consisting of glycine, calcium chloride and sodium chloride; and
(c) 5-50 mg per ml of final container solution of a carbohydrate selected from the group consisting of glucose polymers and sugar alcohols.

5. The composition of claim 4 wherein said protein has a specific activity of at least 99% of theoretical maximum.

6. The composition of any of claims 4 and 5 wherein said carbohydrate is selected from the group consiting of dextran, mannitol and sorbitol

7. The composition of any of claims 4 to 6 wherein said protein is Factor VIII:C.

8. A method of preparing a protein composition, comprising:
(a) purifying a protein to at least 60 % of its theoretical maximum specific activity; and
(b) adding a stabilizer which is a carbohydrate having a molecular weight of 25,000 to 100,000 kD in an amount such that the presence of the stabilizer in the final liquid formulation is 1-50 mg/ml for each 100-300 unit/ml of final container protein activity.

9. The method of claim 8 further comprising the step of free-drying the composition.

10. the method of any of claims 8 and 9 wherein said stabilizer is added to a concentration of 1-5 mg/ml